

Effect of Manure on *Escherichia coli* Attachment to Soil

A. K. Guber,* D. R. Shelton, and Ya. A. Pachepsky

ABSTRACT

Attachment of bacteria to soil is an important component of bacterial fate and transport. *Escherichia coli* are commonly used as indicators of fecal contamination in the environment. Despite the fact that *E. coli* are derived exclusively from feces or manure, effect of the presence of manure colloids on bacteria attachment to agricultural soils was never directly studied. The objective of this work was to evaluate the magnitude of the effect of manure on *E. coli* attachment to soil. *Escherichia coli* attachment to soil was studied in batch experiments with samples of loam and sandy clay loam topsoil that were taken in Pennsylvania and Maryland. *Escherichia coli* cells were added to the water–manure suspensions containing 0, 20, and 40 g L⁻¹ of filtered liquid bovine manure, which subsequently were equilibrated with air-dry sieved soil in different soil to suspension ratios. The Langmuir isotherm equation was fitted to data. Manure dramatically affected *E. coli* attachment to soil. Attachment isotherms were closer to linear without manure and were strongly nonlinear in the presence of manure. The maximum *E. coli* attachment occurred in the absence of manure. Increasing manure content generally resulted in decreased attachment.

THERE IS GROWING CONCERN regarding the potential for contamination of surface and ground water by pathogens from bovine manures. Even though they are considered to be a beneficial fertilizer and soil amendment, bovine manures are a substantial agricultural source of several bacterial human pathogens. *Escherichia coli* O157 and other EHEC strains are commonly found in beef and dairy cattle (Elder et al., 2000). On-farm monitoring of *E. coli* O157:H7 suggests that shedding occurs episodically (up to 10⁵ organisms g⁻¹ feces) and can persist for variable periods of time ranging from 1 to 5 mo (Shere et al., 1998; Zhao et al., 1995). Manure-borne pathogens may enter the soil and travel through vadose zone until they reach ground water (McMurry et al., 1998).

Attachment of bacteria to soil is an important aspect of the bacterial fate and transport, along with straining, mechanical filtration, and size exclusion. Bacteria cell surface properties (Lindqvist and Bengtsson, 1991), properties of soil solid phase (Hagedorn et al., 1978; Lindqvist and Bengtsson, 1991), and soil solution com-

position (Gilbert et al., 1976; Tay et al., 1994; Gannon et al., 1991; Jackson et al., 1994) were listed as factors of the bacteria attachment to soil. High clay content in soil solid phase was reported to promote bacterial adsorption to soil (Hagedorn et al., 1978; Bengtsson, 1989). Jackson et al. (1994) studied the effect of sodium dodecylbenzene sulfonate (DDBS) on *Pseudomonas pseudocaligenes* attachment to silty clay soil. Sodium dodecylbenzene sulfonate enhanced the negative charge associated with the surfactant bacterial complex, resulting in decreased bacterial attachment to soil. Marshall et al. (1971) studied reversible and irreversible sorption of *Achromobacter* R8 onto glass surfaces in the presence MgSO₄ and NaCl solutions of different concentrations. The number of reversibly sorbed bacteria increased with increasing electrolyte charge and concentration.

Organic compounds in soil solutions were shown to affect the attachment of bacteria to minerals. Scholl and Harvey (1992) observed an increase in bacterial adsorption after the removal of dissolved organic carbon from the bacterial suspension used in the adsorption experiment. The authors explained their results by competition between dissolved organic carbon and bacteria for positively charged surface sites on the sand. Johnson and Logan (1996) and Johnson et al. (1996) studied the effect of dissolved organic matter on Savannah River strain A1264 adsorption on quartz and Fe-quartz particles. They concluded that organic matter increased the negative surface charge of quartz and bacteria and made the bacteria–quartz interaction electrostatically unfavorable. Dissolved organic matter has been reported to adsorb to bacterial cell walls and alter their electrophoretic mobility (Gerritson and Bradley, 1987).

Manure-borne bacteria are released to the environment along with manure colloids that can travel alongside with bacteria in soils (Shelton et al., 2003). The sewage-derived organic matter was shown to facilitate the passage of viruses (Pieper et al., 1997) and bacteria (Powelson and Mills, 2001) through sands and gravel. However, both attachment and mechanical retention (i.e., straining) affect the passage, and the effect of the dissolved organic matter on the attachment cannot be deduced from such experiments. Manure colloids are substantially larger than organic molecules and have sizes comparable with bacteria sizes. Their effect on both attachment and mechanical straining may differ from that of dissolved organic compounds. To our knowledge, effect of the presence of manure colloids on bacteria, in particular *E. coli* attachment to agricultural soils, has never been directly studied in a batch experiment.

Escherichia coli is commonly used as an indicator of fecal contamination in the environment. The objective

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Published in J. Environ. Qual. 34:2086–2090 (2005).

Short Communications

doi:10.2134/jeq2005.0039

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Abbreviations: BARC, Beltsville Agricultural Research Center; CFU, colony-forming units.

of this work was to evaluate the magnitude of the effect of manure on *E. coli* attachment to soil in equilibrium batch experiments. The Langmuir isotherm equation was used to quantify and compare *E. coli* attachment in the presence and absence of manure.

MATERIALS AND METHODS

Escherichia coli attachment to the soil was studied in batch experiments. Loam soil samples were taken in 2004 from the A horizon of Tyler soil (fine-silty, mixed, mesic, Aeric Fragiaquolls) in Franklin County, PA. Soil was under a complex mixture of grasses and legumes. Additional sandy loam and clay loam soil samples were taken in 2004 from runoff plots (0–20 cm depth) at the USDA-ARS Beltsville Agricultural Research Center (BARC). Runoff plots were constructed in 1998 using commercial topsoil and planted with blue fescue (*Festuca ovina* L. 'Glaucua') and white clover (*Trifolium repens* L.). Soil texture was measured with the hydrometer method (Gee and Bauder, 1986) after dispersion with sodium pyrophosphate $\text{Na}_4\text{P}_2\text{O}_7$. Particle density was measured with the pycnometer method (Blake and Hartge, 1986). Organic carbon content was measured with the dry combustion method (Nelson and Sommers, 1996). The values of soil pH were measured at a solid to liquid ratio of 1:1 (Thomas, 1996). Selected properties of soil are given in Table 1. The air-dried soil was passed through a 2-mm sieve.

Manure was collected from the Dairy Research Unit of the USDA-ARS facility in Beltsville and stored at a temperature of 4°C for 2 mo prior the experiment. *Escherichia coli* was not detected in manure after 2 mo. Manure contained 15.8% total solids, 1.210 g L⁻¹ of total nitrogen, 0.306 g L⁻¹ of ammonium nitrogen, less than 0.001 g L⁻¹ of nitrate, 0.148 g L⁻¹ of soluble phosphorus, and 0.303 g L⁻¹ of total phosphorus. The manure was filtered through cheesecloth to separate the suspension from plant residue and macroscopic particles. The filtered manure was added to water to obtain water–manure suspensions of 20 and 40 g L⁻¹ liquid manure. The electrical conductivity (EC) of the manure suspension was measured with a Solomat MPM 1000 conductivity meter (Solomat Ltd., Bishops Cleeve, UK). The ionic strength was estimated from values of EC (Griffin and Jurinak, 1973). In 0, 20, and 40 g L⁻¹ manure suspensions, values of pH were 7.21, 8.71, and 8.78, values of EC were 0.0024, 0.576, and 1.062 S m⁻¹, and values of ionic strength were 0.00031, 0.073, and 0.135 mol L⁻¹, respectively.

A wild-type *E. coli*, isolated from bovine manure (BARC dairy herd) was used throughout all experiments. The strain was obtained by plating diluted manure onto MacConkey Agar and incubating overnight at 44.5°C. A single colony with proper morphology was selected and the identity confirmed using a BBL Enterotube II (Becton Dickinson, Sparks, MD). *Escherichia coli* of the same strain and age were used in all experiments. *Escherichia coli* cells were added to the water–manure suspensions containing 0, 20, and 40 mg L⁻¹ of liquid manure. The procedure of the batch experiments was similar to that of Gantzer et al. (2001). Suspensions were added to air-dried soil with soil to suspension ratios of 2:1, 1:1, 1:2, 1:5, and 1:10 and soil mass of 5 or 10 g, with each ratio in triplicates. Soil suspensions were stirred for 30 min followed by centrifugation at 1400 times the acceleration of gravity for 3 min in 50-mL centrifuge tubes (PTD PRO; Elkay, Mansfield, MA). In preliminary experiments, no statistically significant difference ($P > 0.95$) was found between equilibrium concentration after 30 min and 1 h (data not shown). *Escherichia coli* concentrations were measured in the supernatant and applied water–manure suspensions in triplicate. *Escherichia coli* concentra-

Table 1. Selected soil properties.

Soil	Particle density	Clay	Silt	Organic carbon	pH
	Mg m ⁻³				
Tyler loam	2.560	26.4	45.8	3.3	5.61
BARC† loam	2.552	27.0	36.7	2.5	4.65
BARC sandy clay loam	2.611	20.3	26.7	1.7	5.57

† Beltsville Agricultural Research Center.

tions were determined in subsamples of 50 µL volume that had been plated on MacConkey Agar using an Autoplate 4000 spiral platter (Spiral Biotech, Bethesda, MD), and had been incubated for 14 h at a temperature of 42°C. *Escherichia coli* colony-forming units (CFUs) were counted using a Protocol plate reader (Synoptics, Cambridge, UK). The attached *E. coli* cells were calculated from the difference between the amount applied and the amount recovered in the supernatant. Temperature during the experiment was 23°C.

The Langmuir isotherm equation:

$$S = S_{\max} KC / (1 + KC) \quad [1]$$

was fitted to data, where S is the equilibrium concentration of colony-forming units attached to gram of soil (CFU g⁻¹), C is the equilibrium solution concentration of bacteria (CFU L⁻¹), K is the reaction equilibrium constant (L CFU⁻¹), and S_{\max} is the maximum concentration of bacteria attached to soil (CFU g⁻¹).

RESULTS AND DISCUSSION

Manure dramatically affected *E. coli* attachment to soil. Attachment isotherms were closer to linear without manure and were strongly nonlinear in the presence of manure. The maximum *E. coli* attachment occurred in the absence of manure. Increasing manure content generally resulted in decreased attachment (Fig. 1).

The applicability of the Langmuir equation (Eq. [1]) and Langmuir-type equations to bacteria attachment to soil and mineral particle surfaces was shown in previous studies (Fletcher, 1977; Hendricks et al., 1979; Gordon and Millero, 1984). In our study, the values of the coefficient of determination (R^2) for the fits of *E. coli* attachment data to Eq. [1] were sufficiently high to confirm the applicability of this equation (Table 2). The t test showed that all values of R^2 were statistically different from zero at a probability level greater than 0.95. The effects of manure on *E. coli* attachment are reflected by the isotherm parameters (Table 2). The reaction equilibrium constant K was larger in suspensions with manure as compared with suspensions without manure. The maximum equilibrium attachment S_{\max} was substantially smaller in suspensions with manure as compared with suspensions without manure.

A marked solute composition effect on the Langmuir isotherm parameters of bacterial attachment to soil was observed earlier. Hendricks et al. (1979) showed that the Langmuir isotherm parameters for *Staphylococcus aureus* attachment to silt loam soil were influenced by sodium chloride and peptone. The addition of NaCl and peptone caused a substantial reduction in *S. aureus* attachment to the soil resulting in increased K values and a decrease in the values of S_{\max} . The authors concluded that the peptone occupied sites otherwise avail-

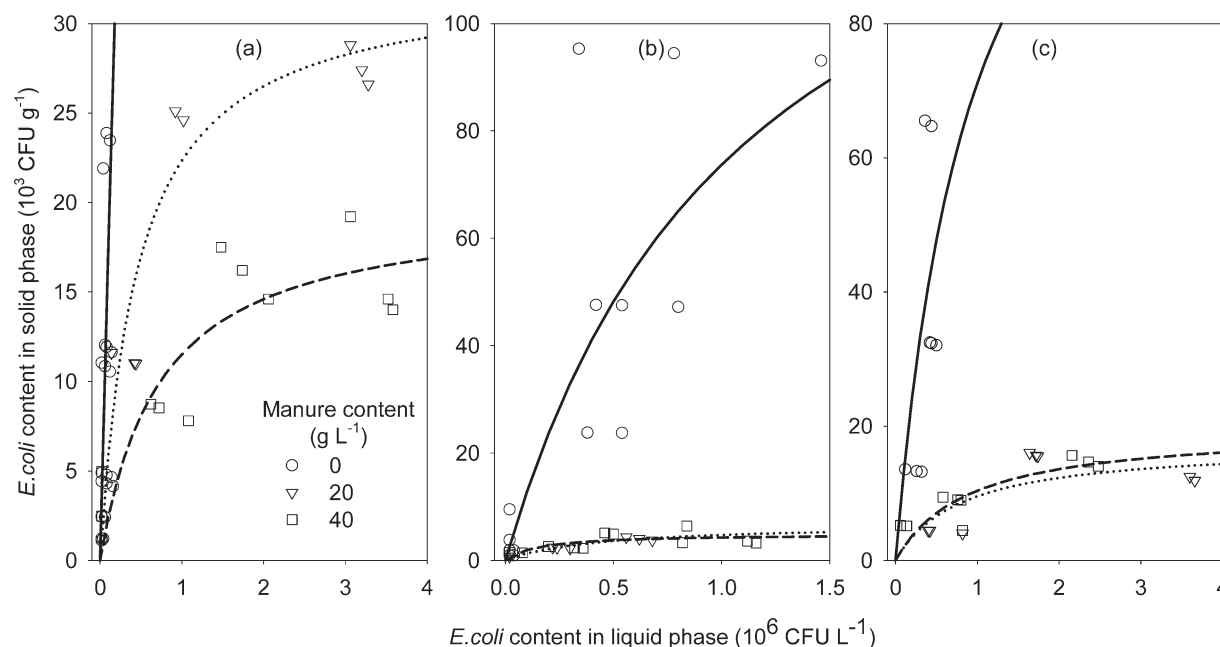


Fig. 1. Attachment isotherms of *Escherichia coli* for (a) Tyler loam soil, (b) Beltsville Agricultural Research Center (BARC) loam, and (c) BARC sandy clay loam. Symbols = measured; lines = fitted Langmuir isotherm equation. CFU, colony-forming units.

able to the bacteria. This supports the hypothesis that manure particulates compete with bacteria for attachment sites on soil.

Manure suspensions had both higher ionic strengths and pH (Table 2) compared with bacteria suspensions in water. Previous studies have shown that increasing ionic strength, due to the increasing solution salinity, causes the increase in attachment of gram-negative bacteria to sand, quartz grains, and silica beads (Marshall et al., 1971; Sharma et al., 1985; Scholl and Harvey, 1992; Fontes et al., 1991; Mills et al., 1994; Johnson et al., 1996). Effect of pH and solution composition on the surface charge and electrophoretic mobility of various strains of *E. coli* has been well documented in literature (i.e., Li and McLandsborough, 1999; Lytle et al., 1999). The effect of ionic strength on the attachment of gram-negative bacteria has been explained by the Deryaguin–Landau–Verwey–Overbeek (DLVO) theory. According to this theory, the initial contact of colloidal particles with a surface is determined by the additive effect of the attractive (van der Waals) and repulsive (electrostatic) forces, and that the balance of these forces may result in adhesion of particles at distance of a few nm from

the surface. Gram-negative bacteria and soil surface are both negatively charged, which makes interaction electrostatically unfavorable. An increase in ionic strength reduces the electrical double layer on both the cells and the mineral surface, allowing the cell to approach the surface within the distance at which van der Waals forces exceed electrostatic repulsion. Solution pH has also been found to affect bacteria attachment to mineral surfaces. Scholl and Harvey (1992) observed that a greater number of *Arthrobacter* sp. were attached to quartz at pH 5.04 than at pH 7.52. The authors hypothesized that an increase in pH resulted in an increase of the negative charge of the quartz surface, such that less negatively charged bacteria cells encountered favorable attachment sites on the quartz surface. Kinoshita et al. (1993) observed a decrease in *P. fluorescens* attachment to silica beads as a result of increase in solution pH from 5.5 to 7. They assumed that hydrophobic factors or specific chemical interactions dominated over the electrostatic repulsion in bacterial adhesion to silica beads. The results of our study indicate that the joint effect of increases in both ionic strength and pH can be quite complex. Assuming the applicability of the DLVO

Table 2. Parameters of the Langmuir isotherm.

Soil	Manure content 0 g L ⁻¹			Manure content 20 g L ⁻¹			Manure content 40 g L ⁻¹		
	<i>K</i> †	<i>S</i> _{max} ‡	<i>R</i> ²	<i>K</i>	<i>S</i> _{max}	<i>R</i> ²	<i>K</i>	<i>S</i> _{max}	<i>R</i> ²
	10 ⁻⁶ L CFU ⁻¹ §	10 ⁴ CFU g ⁻¹		10 ⁻⁶ L CFU ⁻¹	10 ⁴ CFU g ⁻¹		10 ⁻⁶ L CFU ⁻¹	10 ⁴ CFU g ⁻¹	
Tyler loam	0.378	48.2	0.779	2.19	3.26	0.979	1.37	1.99	0.951
BARC¶ loam	0.885	15.7	0.814	2.24	0.683	0.987	6.4	0.491	0.903
BARC sandy clay loam	1.04	13.9	0.807	1.24	1.73	0.929	1.12	1.97	0.946

† Reaction equilibrium constant of the Langmuir isotherm.

‡ Maximum concentration of bacteria attached to soil.

§ CFU, colony-forming units.

¶ Beltsville Agricultural Research Center.

theory, we should conclude that the increase in pH and ionic strength had opposite effects on bacteria attachment in this study, and that the increase in pH mitigated the effect of increasing ionic strength. It should be noted, though, that the DLVO theory has been successfully used to explain colloidal adhesion for shear-free systems, but has failed to predict interaction between cell surface polymers and the solid particle surface (Hendry and Lawrence, 1996; Jucker et al., 1998).

The decrease in bacterial attachment in the presence of manure could also be caused by (i) modification of soil mineral surfaces by soluble manure organic and inorganic constituents; (ii) adsorption of bacteria on manure particulates; (iii) competition of dissolved organic matter and bacteria for adsorption sites; or (iv) modification of bacterial surfaces by dissolved organic matter (Daniels, 1972; Unc and Goss, 2004). No substantial difference in attachment was observed with 20 vs. 40 g L⁻¹ manure in suspensions of BARC soils, whereas the isotherms were different for the two manure concentrations in the Tyler loam soil. The clay content was very similar in all three soils. However, the vegetation cover differed between BARC and Tyler soils. Therefore, the quality and composition of soil organic matter in the solid phase and in the pore solution may be a significant factor in bacteria attachment. It remains to be seen which mechanisms are primarily responsible for the effect of manure on bacterial attachment to soils.

In summary, manure substantially affected the *E. coli* attachment to soil in this study. The Langmuir isotherm parameters provide a convenient way of quantitatively documenting the changes in attachment.

ACKNOWLEDGMENTS

This work was partially supported by the NATO Science Programme, Cooperative Science & Technology Sub-Programme, Collaborative Linkage Grant #979233.

REFERENCES

- Bengtsson, G. 1989. Growth and metabolic flexibility in groundwater bacteria. *Microb. Ecol.* 18:235–248.
- Blake, G.R., and K.H. Hartge. 1986. Bulk density. p. 363–375. *In* A. Klute (ed.) *Methods of soil analysis*. Part 1. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.
- Daniels, S.L. 1972. The adsorption of microorganisms onto solid surfaces, a review. *Dev. Ind. Microbiol.* 13:211–253.
- Elder, R.O., J.E. Keen, G.R. Siragusa, G.A. Barkocy-Gallagher, M. Koohmaraie, and W.W. Laegreid. 2000. Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. *Proc. Natl. Acad. Sci. USA* 97:2999–3003.
- Fletcher, M. 1977. The effects of culture concentration and age, time, and temperature on bacterial attachment to polystyrene. *Can. J. Microbiol.* 23:1–6.
- Fontes, D.E., A.L. Mills, G.M. Hornberger, and J.S. Herman. 1991. Physical and chemical factors influencing transport of microorganisms through porous media. *Appl. Environ. Microbiol.* 57:2473–2481.
- Gannon, J., U. Mingelgrin, M. Alexander, and R.J. Wagenet. 1991. Bacterial transport through nonhomogenous soil. *Soil Biol. Biochem.* 23:1155–1160.
- Gantzer, C., L. Gillerman, M. Kuznetsov, and G. Oron. 2001. Adsorption and survival of faecal coliforms, somatic coliphages and F-specific RNA phages in soil irrigated with wastewater. *Water Sci. Technol.* 43:117–124.
- Gee, G.W., and J.W. Bauder. 1986. Particle analysis. p. 383–412. *In* A. Klute (ed.) *Methods of soil analysis*. Part 1. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.
- Gerritsen, J., and S.W. Bradley. 1987. Electrophoretic mobility of natural particles and cultured organisms in freshwaters. *Limnol. Oceanogr.* 32:1049–1058.
- Gilbert, R.G., C.P. Gerba, R.C. Rice, H. Bouwer, C. Wallis, and J.L. Melnick. 1976. Virus and bacteria removal from wastewater by land treatment. *Appl. Environ. Microbiol.* 32:333–338.
- Gordon, A.S., and J. Millero. 1984. Electrolyte effects on attachment of an estuarine bacterium. *Appl. Environ. Microbiol.* 47:495–499.
- Griffin, R.A., and J.J. Jurinak. 1973. Estimation of activity coefficients from the electrical conductivity of natural aquatic systems and soil extracts. *Soil Sci.* 116:26–30.
- Hagedorn, C., D.T. Hansen, and G.H. Somonson. 1978. Survival and movement of fecal indicator bacteria in soil under conditions of saturated flow. *J. Environ. Qual.* 7:55–59.
- Hendricks, D.W., F.J. Post, and D.R. Khairnar. 1979. Adsorption of bacteria on soils: Experiment, thermodynamic rationale, and application. *Water Air Soil Pollut.* 12:219–232.
- Hendry, M.J., and J.R. Lawrence. 1996. Transport of bacteria through geologic media. *Can. J. Microbiol.* 42:410–422.
- Jackson, A., D. Roy, and G. Breitenbeck. 1994. Transport of a bacterial suspension through a soil matrix using water and an anionic surfactant. *Water Res.* 28:943–949.
- Johnson, W.P., and B.E. Logan. 1996. Enhanced transport of bacteria in porous media by sediment-phase and aqueous-phase natural organic matter. *Water Res.* 30:923–931.
- Johnson, W.P., M.J. Martin, M.J. Gross, and B.E. Logan. 1996. Facilitation of bacterial transport through porous media by changes in solution and surface properties. *Colloids Surf. A* 107:263–271.
- Jucker, B.A., J. Alexander, B. Zehnder, and H. Harms. 1998. Quantification of polymer interactions in bacterial adhesion. *Environ. Sci. Technol.* 32:2909–2915.
- Kinoshita, T., R.C. Bales, M.T. Yahya, and C.P. Gerba. 1993. Bacteria transport in a porous medium: Retention of *Bacillus* and *Pseudomonas* on silica surfaces. *Water Res.* 27:1295–1301.
- Li, J., and L.A. McLandsborough. 1999. The effects of the surface charge and hydrophobicity of *Escherichia coli* on its adhesion to beef muscle. *Int. J. Food Microbiol.* 53:185–193.
- Lindqvist, R., and G. Bengtsson. 1991. Dispersal dynamics in groundwater bacteria. *Microb. Ecol.* 21:49–71.
- Lytle, D.A., E.W. Rice, C.H. Johnson, and K.R. Fox. 1999. Electrophoretic mobilities of *Escherichia coli* O157:H7 and wild-type *Escherichia coli* strains. *Appl. Environ. Microbiol.* 65:3222–3225.
- Marshall, K.C., R. Stout, and R. Mitchell. 1971. Selective sorption of bacteria from seawater. *Can. J. Microbiol.* 17:1413–1416.
- McMurry, S.W., M.S. Coyne, and E. Perfect. 1998. Fecal coliform transport through intact soil blocks amended with poultry manure. *J. Environ. Qual.* 27:86–92.
- Mills, A.L., J.S. Herman, G.M. Hornberger, and T.H. DeJesus. 1994. Effect of solution ionic strength and iron coating on mineral grains on the sorption of bacterial cells to quartz sand. *Appl. Environ. Microbiol.* 60:3300–3306.
- Nelson, D.W., and L.E. Sommers. 1996. Total carbon, organic carbon, and organic matter. p. 961–1011. *In* D.L. Sparks (ed.) *Methods of soil analysis*. Part 3. SSSA Book Ser. 5. SSSA, Madison, WI.
- Pieper, A.P., J.N. Ryan, R.W. Harvey, G.L. Amy, T.H. Illangasekare, and D.W. Metge. 1997. Transport and recovery of bacteriophage PRD1 in a sand and gravel aquifer: Effect of sewage-derived organic matter. *Environ. Sci. Technol.* 31:1163–1170.
- Powelson, D.K., and A.L. Mills. 2001. Transport of *Escherichia coli* in sand columns with constant and changing water contents. *J. Environ. Qual.* 30:238–245.
- Scholl, M.A., and R.W. Harvey. 1992. Laboratory investigations on the role of sediment surface and groundwater chemistry in transport of bacteria through a contaminated sandy aquifer. *Environ. Sci. Technol.* 26:1410–1417.
- Sharma, M.M., Y.I. Chang, and T.F. Yen. 1985. Reversible and irreversible surface charge modification of bacteria of facilitating transport through porous media. *Colloids Surf.* 16:193–206.
- Shelton, D.R., Y.A. Pachepsky, A.M. Sadeghi, W.L. Stout, J.S. Karns, and W.J. Gburek. 2003. Release rates of manure-borne coliform

- bacteria from data on leaching through stony soil. *Vadose Zone J.* 2:34–39.
- Shere, J.A., K.J. Bartlett, and C.W. Kaspar. 1998. Longitudinal study of *Escherichia coli* O157:H7 dissemination on four dairy farms in Wisconsin. *Appl. Environ. Microbiol.* 64:1390–1399.
- Tay, Y., J.T. Gannon, P. Baveye, and M. Alexander. 1994. Transport of bacteria in an aquifer sand-experiments and model simulations. *Water Resour. Res.* 31:3243–3252.
- Thomas, G.W. 1996. Soil pH and soil acidity. p. 475–490. *In* D.L. Sparks (ed.) *Methods of soil analysis*. Part 3. SSSA Book Ser. 5. SSSA, Madison, WI.
- Unc, A., and M.J. Goss. 2004. Transport of bacteria from manure and protection of water resources. *Appl. Soil Ecol.* 25:1–18.
- Zhao, T., M.P. Doyle, J. Shere, and L. Garber. 1995. Prevalence of enterhemorrhagic *Escherichia coli* O157:H7 in a survey of dairy herds. *Appl. Environ. Microbiol.* 61:1290–1293.